

## Circulating MicroRNAs As Potential Biomarkers of Coronary Artery Disease A Promise to Be Fulfilled?

Riccardo Contu, Michael V.G. Latronico, Gianluigi Condorelli

Atherosclerotic coronary artery disease (CAD) continues to be an important socioeconomic problem and the leading cause of death in the Western world. Several cellular components, including endothelial, inflammatory, and smooth muscle cells of the arterial wall, participate in the generation of an atherosclerotic plaque,<sup>1</sup> the growth of which is responsible for a progressive narrowing of the lumen of the affected coronary vessel and the ensuing decrease in oxygen-rich blood flow to downstream heart muscle, which may then be acutely or chronically damaged.

Progression of CAD is highly variable and is driven by both environmental factors and genetic determinants. The genetic risk is thought to be attributable to an unfavorable combination of genetic variations in multiple genes that have not yet been completely characterized. However, major independent risk factors for CAD are known to include familiarity, high blood cholesterol levels, hypertension, smoking, diabetes, and obesity. Innovative early and reliable biomarkers and therapeutic targets for CAD would thus be acclaimed.

MicroRNAs (also known as miRs or miRNAs) are approximately 20- to 25-nt-long noncoding RNAs that negatively regulate gene expression by binding to sites in the 3' untranslated region of targeted messenger RNAs.<sup>2</sup> These small RNAs have been found to be involved in almost every biological process, from cellular differentiation and proliferation<sup>3,4</sup> to cell death and apoptosis,<sup>5,6</sup> from synaptic plasticity<sup>7</sup> to immunity<sup>8</sup> and cardiovascular development<sup>9</sup>; in addition, changes in miRNA levels and activity have been linked to human pathologies, including cancer<sup>10</sup> and cardiovascular diseases.<sup>11,12</sup>

Recently, miRNAs were found to be present in the circulating bloodstream,<sup>13–15</sup> together with other types of noncoding RNA, DNA, and mRNA.<sup>16</sup> In fact, despite the high sensibility of all ribonucleic acids to RNase activity, it was discovered that plasma and serum miRNAs possess robust stability even after cycles of freezing/thawing.<sup>14,15</sup> Moreover, circulating miRNAs were found to vary significantly during

pregnancy<sup>13,15</sup> and in the presence of several types of cancer.<sup>17</sup> Along these lines, recent reports<sup>18,19</sup> showed that after acute myocardial infarction in humans and mice, muscle-enriched miRNAs, such as miR-1, miR-133a, miR-133b, and miR-499-5p, are increased in plasma.

The diagnostic and prognostic value of miRNA blood levels is currently exploited in cancer (reviewed elsewhere<sup>17</sup>), although results are not always coherent; in this type of disease, miRNA profiling is usually carried out comparing serum with tissue expression. Tissue biopsy, which is a fundamental step for disease staging in cancer, is however a more critical undertaking in most cardiovascular pathologies, making the validation of serum miRNA as a disease biomarker more complex than cancer.

In this issue of *Circulation Research*, Fichtlscherer et al<sup>20</sup> address the relation between levels of circulating miRNAs and CAD in humans, exploring the power of circulating miRNA as biomarkers for CAD. Using a high-throughput array to profile the circulating miRNA signature in a small study group (n=8 patients with CAD versus n=8 healthy volunteers), the authors identified 46 significantly downregulated miRNAs and 20 significantly upregulated miRNAs in the plasma of CAD patients. Some of the downregulated miRNAs were then selected for further analysis: their level was measured by quantitative real-time PCR in 2 larger, distinct groups (a derivation cohort comprising n=36 CAD patients versus n=17 healthy controls and a validation cohort comprising n=31 CAD patients versus n=14 healthy controls). The circulating levels of endothelial-expressed miR-126, miR-92a, and miR-17<sup>21–23</sup> were confirmed to be significantly reduced in patients with CAD compared to healthy controls, although there were small differences between the cohorts, probably on account of the different sources used (ie, plasma for the derivation cohort and serum for the validation cohort) and the different quantification methods used. In addition, both the vascular smooth muscle enriched miR-145 (but not the cotranscribed miR-143)<sup>24,25</sup> and the inflammatory cell-related miR-155<sup>26</sup> were found to be significantly downregulated in patients with CAD. In contrast, a trend toward increased blood levels of cardiac-enriched<sup>11</sup> miR-208a and miR-133a in CAD patients was detected. Interestingly, some of the circulating miRNAs found downregulated in CAD patients originate from the cellular components of the vasculature (endothelial, inflammatory, and smooth muscle cells), known to play a key role in both onset and progression of atherosclerosis, suggesting a pathophysiological role of these miRNAs in human CAD. Moreover, reduced expression of endothelial miRNAs in the blood of CAD patients may also

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**Non-standard Abbreviations and Acronyms**

<b>CAD</b>	coronary artery disease
<b>miR</b>	microRNA
<b>miRNA</b>	microRNA

reflect a decrease in the number of circulating endothelial cell progenitors, shown by the same authors to occur in patients with CAD.<sup>27</sup>

The limitations of this study include the small number of individuals making up the cohorts and the differences in key parameters of the cohorts that may affect miRNA levels, such as age, sex, and pharmacological treatments. In addition, only a few of the 20 circulating miRNAs initially found to be significantly upregulated in the CAD group were further validated in larger cohorts, thus leaving room for future analysis.

In conclusion, circulating miRNAs may become helpful and reliable tools for the diagnosis and prognosis of patients with CAD or other cardiovascular diseases. However, only future multicentric investigations based on much larger and multiethnic patient populations, and using standardized procedures for sample processing and RNA extraction, will tell us whether miRNAs will fulfill their promise as CAD biomarkers.

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